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The 10th Annual

Plant Development Workshop

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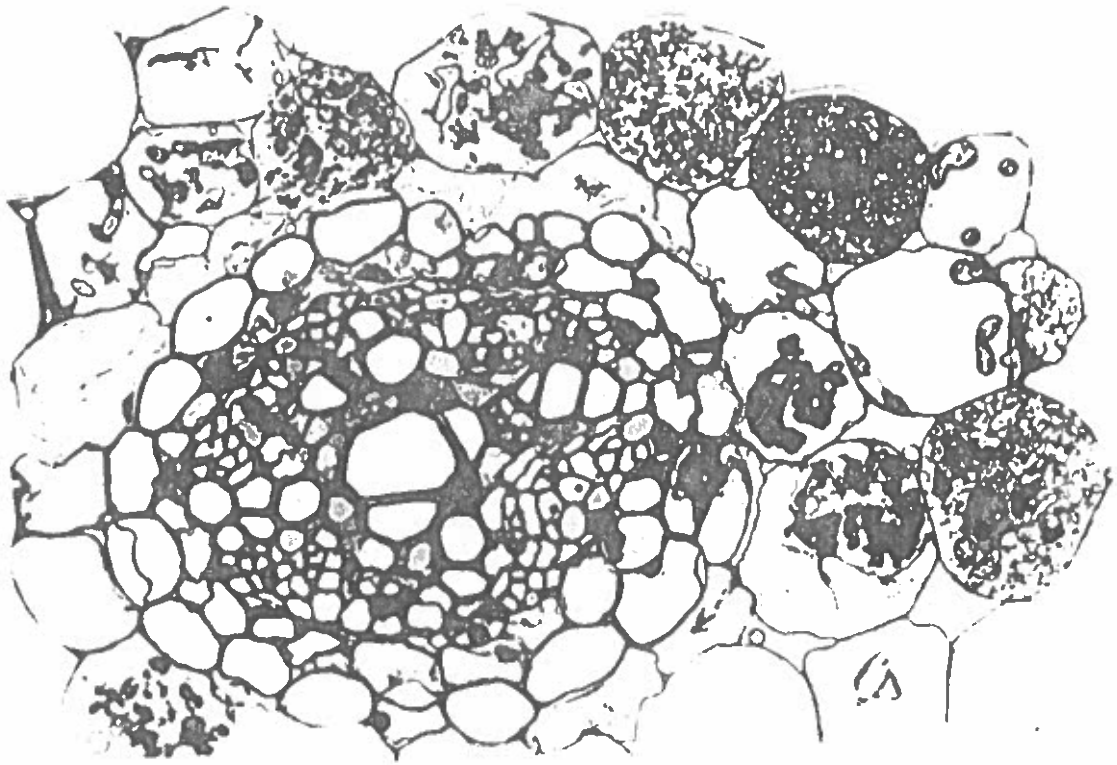
University of Guelph



10th Plant Development Workshop

Room 103 University Centre

University of Guelph

Sat. Apr. 17, 1982

8:30 - 9:30 Registration and Coffee
9:30 - 9:40 Introductory remarks and announcements - R.L. Peterson

9:40 - 10:55 Contributed papers - Chairperson - A. Smith

9:40 - 9:55 FOLIAR RESPONSE OF ARTEMESIA TILESII TO SIMULATED ACID RAIN. Adams, C.M., N.G. Dengler, T.C. Hutchison, Department of Botany, University of Toronto

Experiments were performed to investigate macroscopic and microscopic injury to leaf tissues of clones of Artemesia tilesii by simulated acid rain. This species shows remarkable tolerance to SO_2 and acid aerosol at the Smoking Hills, N.W.T. where it grows in an area heavily fumigated with SO_2. Evidence from greenhouse experiments with simulated acid rain have shown that clones of A. tilesii from the Smoking Hills and from an unpolluted site in Alaska have a greater overall tolerance to acid rain than most herbaceous species. Macroscopically visible injury occurred when plants were exposed to rainfall of pH 3.0 and below. Leaf clearings and scanning electron microscopy showed the existence of cellular damage at pH 3.5 in the absence of macroscopic foliar injury. SEM examination revealed significant differences in stomatal aperture size between plants treated with acidified rain solutions and those treated with rain of pH 5.6 (control). Lesion development in A. tilesii was studied by sectioning plastic embedded tissue after 1, 2, 4 or 8 exposures to simulated rain at pH levels of 5.6, 3.0 and 2.5. Results suggest that most lesions develop from an initial collapse of a small number of adaxial epidermal cells and that there is a progressive injury of tissue layers in the direction of the abaxial surface. Hyperplasia and hypertrophy occurred in palisade and spongy mesophyll cells prior to cell collapse. In a small percent of lesions observed for Artemesia tilesii from the Alaskan population hypertrophy and hyperplasia occurred in the mesophyll without collapse of the overlying epidermal cells.

9:55 - 10:10 DEVELOPMENTAL PATTERNS IN HYDROCHARIS MORSUS-RANAE. R.W. Scribailo. Department of Botany and Genetics University if Guelph.

The Hydrocharitaceae is a family composed of some fifteen genera, each of which shows greater or lesser degrees of adaptation to an aquatic environment. Hydrocharis is a genus in the family and consists of three species. The most widespread of these, Hydrocharis morsus-ranae was introduced from Europe into Canada at the Central Experimental Farm in Ottawa in 1932 and has been spreading through the St. Lawrence and Rideau River systems since that time. The species disperses by means of rapid turion production in the fall and can quickly clog shallow water bodies with its extensive stoloniferous growth from these overwintering buds in the spring. The present study of Hydrocharis morsus-ranae has been undertaken to examine several different aspects of the morphology and ecology of this species. The major part of this work will involve looking at the vegetative and floral development of the species from the time of inception of primordia through their maturation process to either stolons or flowers. Floral and vegetative material was examined by an epi-illumination microscopy technique and by SEM. Present work to date has revealed that the vegetative branching pattern is such that stolon primordia arise every second leaf, resulting in a monopodial branching system. This type of rapid stolon production in part explains the ability of the plant to spread extensively by vegetative means. Despite this capacity for vegetative spread, the species still flowers profusely in the wild. It is generally accepted that seed set and germination are not important in maintenance and dispersal of Hydrocharis morsus-ranae populations, though this has never really been examined in detail.

10:10 - 10:25 DEVELOPMENT OF THE DIMORPHIC LEAVES OF PELLIONIA DAVEAUANA. Petra A. Mueller, Nancy G. Dengler. Department of Botany, University of Toronto.

The plagiotropic shoot system of Pellionia daveauana bears opposite pairs of large ventral and small dorsal leaves. The ovate ventral leaves average 2.4 cm in length while the peg-like dorsal leaves rarely exceed 0.2 cm in length. The anatomical organization of both leaf types is similar although there are fewer tissue layers in the dorsal leaves. This species was selected to test Goebel's hypothesis of developmental arrest since the normal foliage leaf (ventral) and the reduced leaf (dorsal) are produced simultaneously on the same shoot apex. Observations of growth rates indicate that the average time for expansion is similar for both leaf types although the rate of growth was greater in

the ventral leaves. Scanning electron microscopy of whole shoot apices and light microscopy of serial cross sections of embedded shoot tips indicated that the two types of primordia can be distinguished morphologically shortly after inception. Development of the ventral leaf also differs from the dorsal by extensive plate meristem activity which gives rise to a broad ovate lamina. The cessation of cell division and conspicuous cell enlargement and vacuolation occur at earlier developmental stages in the dorsal leaf. If the dorsal leaf represents an arrested form of the ventral leaf, then patterns of initiation and the progress of cell division and enlargement would be expected to be similar through maturation of the reduced leaf types. Our observations indicate that the developmental pathways of the dorsal and ventral leaves differ from inception and therefore do not support Goebel's hypothesis.

10:25 - 10:40 INTERACTIONS BETWEEN THE MYCORRHIZAL FUNGUS GLOMUS EPIGAEUS DANIELS & TRAPPE AND ASPARAGUS OFFICINALIS L. ROOTS. R.B. Hussey, R.L. Peterson. Department of Botany and Genetics, University of Guelph.

Asparagus officinalis L. roots have a well-defined exodermis consisting of alternating short and long cells which result from asymmetric cytokinesis of ground meristem cells. Although both cell types become suberized, the short cells undergo this process more slowly. Hyphae of Glomus epigaeus Daniels & Trappe penetrate the epidermis directly but only enter the short cells of the exodermis. An intracellular hyphal coil is produced in the short cell, after which hyphae penetrate to the inner cortical region where arbuscules are formed. The structural characteristics of the short and long cells in relation to mycorrhizal development are examined by fluorescence, bright field and transmission electron microscopy.

10:40 - 10:55 EFFECT OF SECONDARY GROWTH ON THE CONFORMATION AND PERMEABILITY OF THE ENDODERMIS OF BEAN (VICIA FABA), SUNFLOWER (HELIANTHUS ANNUUS) AND GARDEN BALSAM (IMPATIENS BALSAMINA). Carol Ann Weerdenburg, Carol Peterson. Department of Biology, University of Waterloo.

Roots undergoing secondary growth exhibit massive girth increases due to the activity of the vascular cambium arising in the pericycle. To determine the effects of stelar girth increase on the endodermis, cleared cross sections from areas of primary and secondary growth in bean, sunflower and garden balsam roots were examined. The sections were stained with Chelidonium majus root extract and viewed with ultra-violet light using an epifluorescence microscope. Endodermal cell counts, Casparian band lengths and endodermal diameters increased dramatically in the area

of secondary growth. The permeability of the apoplast in roots undergoing secondary growth was tested by treatment with 0.01% Calcofluor White M2R for 24h. The Casparian band continued to function as an effective barrier to apoplastic movement despite the increase in endodermal cell size and number. Decortication with concurrent disruption of the endodermis resulted in massive penetration of the dye into the stele. Roots with mature periderm were also freely permeable to Calcofluor.

10:55 - 11:15 Coffee

11:15 - 12:15 Contributed papers - Chairperson - B. Hussey

11:15 - 11:30 CHROMOSOME ELIMINATION IN CAFFEINE-INDUCED BINUCLEATE CELLS IN ROOTS OF A DIPLOID INTERSPECIFIC HYBRID OF HORDEUM. W.G. Wheatley, Crop Science Department, University of Guelph.

In root meristems of diploid interspecific hybrids (vulgare-bulbosum) of Hordeum spontaneous chromosome elimination occurs at a relatively low rate. In an attempt to distinguish whether chromosome elimination in these cells is prompted by nuclear or cytoplasmic factors, chromosome elimination was studied in binucleate cells induced by treatment with 1% caffeine. Bimitotic cells and recently divided products of bimitotic cells were scored by observing whether neither mitosis, one mitosis or both mitoses within the same cell exhibited or had recently undergone chromosome elimination. In the bimitotic cells, chromosome elimination was observed in only one of the two mitoses with a frequency of 0.159 whereas both mitoses exhibited chromosome elimination with a frequency of 0.024. The occurence of chromosome elimination in one mitosis appears to be independent of the response of the other mitosis within the same cell. Since the two nuclei of a binucleate cell share a common cytoplasm throughout their interphase and appear to pass through their mitotic phases synchronously, the results suggest that the factors which prompt chromosome elimination in the hybrid cells are inherent to the hybrid nuclei rather than the cytoplasm.

11:30 - 11:45 POSSIBLE REVERSION TO AN ANCESTRAL PHENOTYPE IN MAIZE FOLLOWING FLOODING AT LOW TEMPERATURE. Stewart Rood, Richard Pharis, David Major. Faculty of Forestry, University of Toronto, Department of Biology, University of Calgary, and Agriculture Canada Research Station, Lethbridge.

Following flooding at low temperature early in the life-cycle of four diverse maize genotypes, the phenotype at anthesis was dramatically altered. Plants i) tillered profusely, ii) showed reduced internode elongation, iii) produced terminal inflorescences with both male and female flowers and iv) contained large floral bracts. The resultant phenotype was thus very similar to that of an ancestral form which has previously been reconstructed. This possible

reversion provides circumstantial evidence that the genes responsible for the primitive pod corn traits have not been eliminated from modern maize genotypes, but rather are repressed under normal environmental conditions. However, the probable involvement of "downy mildew" (possibly from Sclerophthora macrospora - "crazy top") complicates this interpretation.

11:45 - 12:00 NUCLEAR RNA CONTENT OF CAFFEINE INDUCED BINUCLEATE CELLS IN EXCISED ROOTS OF PISUM SATIVUM. S.W. Armstrong, Department of Biology, McMaster University.

Roots of four day old pea seedlings were excised and grown in culture for three days. Roots were treated with 5-aminouracil, to produce a synchronized wave of cells in mitosis, and the synchronized mitotic cells were then treated with 0.1% caffeine for 1 hr to induce binucleate cell formation. It has previously been shown, for binucleate cells induced in this manner, that by 2 hr after caffeine treatment, the two sister nuclei have a mean size ratio of 1.19 : 1 (larger : smaller) and a mean ratio of protein content of 1.14 : 1. Furthermore, over one cell cycle in the binucleate cells, the correlation between mean nuclear volume and mean protein content was 0.79. This suggests that proteins make a significant contribution to nuclear volume. The experiment reported here was carried out to estimate the contribution of RNA to nuclear size. Roots fixed 2 hr after caffeine treatment were stained with gallocyanin - chromalum. This stains DNA and RNA: half of the preparation was treated with RNase to remove RNA, and nucleic acid content was determined by microspectrophotometry. RNA content was obtained by subtracting DNA content from total nucleic acid content. The binucleate cells studied were all at G_1 (Armstrong and Davidson, 1982). The mean ratio of total nucleic acid content of sister nuclei was 1.14 : 1. However, RNA makes up only 10% of total nucleic acids and though the ratio of RNA content was 1.9 : 1 in sister nuclei it is suggested that it makes only a minor contribution to the determination of nuclear volume. Functionally, however, the differences in RNA may play a much larger role in controlling the behaviour of the nuclei. The large asymmetry in the RNA content of sister nuclei itself is evidence of differential behaviour of nuclei, even when sharing a common cytoplasm.

12:00 - 12:15 IN VITRO ANTHER CULTURE IN ASPARAGUS OFFICINALIS. Christine Deslandes, Department of Horticultural Science, University of Guelph.

Attempts were made to recover haploid plants from Asparagus officinalis by means of anther culture. Experiments were

conducted with the cultivar Viking which is best adapted to our growing conditions. Production of callus from the anthers was increased substantially when the plated anthers were incubated in the dark for the first weeks of culture. Callus yields were increased further when anthers were excised from flower buds which had been cold pretreated (7 C $\pm$ 2) for 2 to 6 days. Cytological examinations of plants regenerated from callus revealed a wide variety of ploidy levels. Several aneuploids were found as well as diploids and tetraploids; no haploids were discovered. Examinations of plated anthers seem to indicate that callus was produced from the microspores. No proliferation of the somatic cells of the anthers was observed.

12:15 - 1:45 Lunch and Posters

1:45 - 4:30 "Use of Cell and Tissue Culture Systems in Studies of Differentiation and Development in Plants"
Chairperson - K. Kasha

1:45 - 2:00 SHOOT TIP CULTURE OF RED CLOVER (TRIFOLIUM PRATENSE L.) C. Campbell, D.T. Tomes. Department of Crop Science, University of Guelph.

Storage and rapid propagation of valuable red clover genotypes using shoot tip culture would be useful for breeder seed increase, hybrid seed production and parental plant maintenance for breeding and genetic studies. Following excision and surface sterilization, shoot tips from crown buds are placed horizontally on multiplication medium (Gamborgs B-5 medium with the cytokinin benzyladenine at 2.0 mg/l). After four weeks on multiplication medium a mean of 6.9 shoots/explant was observed. Significant genotypic differences in the number of shoots produced on multiplication medium were noted. The multiple shoots are separated and subcultured on either multiplication medium for continued propagation or maintenance medium (B-5 medium without hormones) for the rapid development of a single shoot. Once on maintenance medium, a plant can be stored at 4^{o}C for at least eight months or transferred to soil.

Plants propagated by shoot tip culture were identical to the plants from which they were derived for flower colour, leaf markings, leaf shape and disease reaction. A field study compared plants from shoot tip culture and plants from cuttings. Seed yield and first year forage yield were similar for both, while shoot tip culture derived plants had significantly better winter hardiness and higher second year yield.

2:00 - 2:15 COMPARISON OF PLANTS DERIVED FROM CUTTINGS, NODE CULTURES AND EMS-TREATED NODE CULTURES OF BIRDSFOOT TREFOIL. B. Orshinsky and D.T. Tomes. Department of Crop Science, University of Guelph.

Node cultures of six genotypes of birdsfoot trefoil (Lotus corniculatus L.) were exposed to 0.5% (v/v) ethyl methanesulfonate (EMS) for 8 h and transferred to regeneration medium. Untreated nodes were similarly subcultured. Twenty-three days after mutagenesis, 72-86% of the EMS-treated nodes had produced shoots, compared to 98-100% for the untreated nodes. Survival upon transfer to the growth room was lower for plants derived from treated vs. untreated nodes for four of the six genotypes.

Plants regenerated from mutagenized and non-mutagenized nodes were transplanted to the field along with plants of each genotype that had been propagated as asexual cuttings. At the time of first flower, each plant was examined for a number of morphological traits. Analysis of variance revealed significant genotypic differences. Averaged over genotypes, source of plant material was also highly significant for most traits. Plants from mutagen-treated node cultures were significantly later in flowering but otherwise mutagen treatment had little apparent effect. Plants from cultured nodes (mutagenized and non-mutagenized) differed from cutting-derived plants by being slightly shorter and having more branches and shorter, broader leaflets. When whole plant dry weight was determined at the end of the growing season, plants derived from untreated node cultures displayed significantly higher yield than those from cuttings. Aside from a number of induced chlorophyll mutations, there was no definite indication of genetic changes in plants from either the mutagenized or non-mutagenized cultures; rather, the observed variation was most likely due to the hormonal conditions of the tissue culture system. The presence of recessive mutations cannot be ruled out, but intercrossing of the regenerated plants would be required in order to detect such mutations.

2:15 - 2:30 IN VITRO MORPHOGENESIS FROM TISSUES, CELLS AND PROTOPLASTS OF SOLANUM MELONGENA. S. Gleddie, W.A. Keller, G. Setterfield. Department of Biology, Carleton University and Agriculture Canada, Ottawa Research Station.

Physiological parameters influencing morphogenesis in cultured tissues, cells and protoplasts of eggplant were examined. Of five auxins tested, only NAA supplied in Murashige and Skoog medium induced somatic embryogenesis on leaf explants. The optimal embryogenic response was obtained at 10 mg/l NAA and this concentration of auxin did not inhibit embryo maturation. Both cytokinins and

ABA inhibited NAA-induced somatic embryogenesis. NH_4^+ was essential in the medium and an optimal NO_3^- / NH_4^+ ratio of 2/1 was established in the total nitrogen content at 60 mM. Each of seven cultivars tested was embryogenic although there were quantitative differences in their responses. Shoot organogenesis on leaf explants was promoted by either of the cytokinins zeatin, BAP, 2iP or kinetin. Excised embryos or shoots gave rise to mature plants after transfer to hormone-free media. Protoplasts isolated from embryogenic cell suspensions were cultured in Nagata and Takebe medium and shown to undergo reformation of cell walls, division and formed cell colonies. Plant regeneration from these colonies was achieved through kinetin-induced shoot organogenesis.

2:30 - 3:00 PLANT CELL CULTURES AND METABOLIC DIFFERENTIATION
B.E. Ellis, Department of Chemistry, University of Guelph.

3:00 - 3:15 COFFEE

3:15 - 3:45 VARIATIONS OF CALLUS CULTURE LINES OF BIRDSFOOT TREFOIL IN RELATION TO IN VITRO SELECTION, CALLUS GROWTH RATE, AND SHOOT DIFFERENTIATION. D.T. Tomes and B.R. Orshinsky. Deparment of Crop Science, University of Guelph.

Callus culture lines are very useful for studies of development, in vitro selection, and plant regeneration. Our primary objective in the tissue culture of birdsfoot trefoil is in assisting the plant breeding program. However, the observation of tissue culture variation has been interesting on its own merit and relevant to our primary objective. Our research has shown wide variation for callus growth rate, root and shoot regeneration, callus colour and organization, and differential response to plant growth regulators.

Although most of our studies have been in the cultivar Leo, large differences in callus growth rates are observed between genotypes. There does not seem to be any relationship between yield of the plant and its callus growth rate, contrary to some reports in the literature. Shoot differentiation, usually by organogenesis, is conditioned by the genotype of the parent, the culture media used and the volume of callus used. Callus that is finely divided by mild homogenization usually differentiates more rapidly than non-homogenized callus. Differentiation ability deteriorates with time in culture.

Homogenization of callus followed by plating on agar may be a useful technique for in vitro selection. Observations indicate that specific culture lines vary in their response to specific selective agents. So far, there does not appear to be a consistent method to predict the utility of a culture line for selection with

easily observable traits such as callus colour, growth rate, or structure. Regenerated plants from such selection experiments will reveal the value of this type of selection in birdsfoot trefoil. The observation of plants with altered response to plant growth regulators as a by-product of selection experiments may prove useful.

3:45 - 4:00 MICROTUBULE (MT) ORGANIZATION AND CELL SHAPE IN CULTURED PLANT CELLS. G. Setterfield, D. Simmonds, M. Tanchak, D.L. Brown, K. Rogers. Departments of Biology, Carleton University and University of Ottawa.

Vicia hajastana (vetch) cells cultured with 2,4D show coupled cell expansion and division and maintain isodiametric shape with diameters of 30 - 50 μm. On transfer from 2,4D to GA the cells cease division but continue elongating, reaching lengths of over 600 um. Immunofluorescent staining using anti-tubulin shows that interphase cells in 2,4D have relatively low numbers of mt with only approximate transverse orientation. These cortical mt disappear during mitosis, where all mt are found in the spindle or phragmoplast. Elongating cells in GA show numerous transversely oriented mt, paralleling cellulose microfibril orientation, which increase in number as cell length increases. Colchicine applied with GA disrupts mt and the cells grow isodiametrically. Cells removed from colchicine after 16-30h continue to grow isodiametrically and form only relatively small numbers of mt with variable degrees of order. Cells treated with colchicine after 3-4 days in GA maintain elongation. These results indicate that mt's have differing degrees of stability at different stages of cell development, which may be important in establishing and maintenance of plant cell form.

4:00 - 4:30 ORGAN CULTURE. D. Greyson. Department of Plant Sciences, University of Western Ontario.

4:30 - REFRESHMENTS

POSTERS

AN ANALYSIS OF GROWTH REGULATORY SUBSTANCES IN DEVELOPING PEA ROOT NODULES
Guy Charbonneau, William Newcomb, Department of Biology, Queen's University.

Root nodules in legumes are induced by the infection of roots by a bacterium of the genus Rhizobium. That hormonal substances are implicated in the nodulation process has been suggested by considerable evidence: root nodules produce higher levels of extractable hormones than other root tissues; Rhizobium excrete auxins and cytokinins in vitro; similar cortical cell polyploid mitoses and enlargement occurs in cortical explants cultured with cytokinins and auxins and developing root nodules.

The relative contribution of growth regulatory substances by each member of the symbiosis is presently unknown. Solvent extractions of tissues and subsequent gas liquid chromatography (GLC) were used to measure the levels of hormonal substances such as auxins, cytokinins and abscisic acid, based on co-chromatography with authentic standards. Tissues of uninfected roots, developing root nodules at 14, 21, 28 and 35 days and cultures of Rhizobium leguminosarum were analysed.

Preliminary results suggest that auxin concentrations are higher in root nodules than in comparable non-nodulated root tissues. As nodules grow and mature, auxin levels apparently decline.

NOVEL "ORGANELLES" IN RHIZOBIUM INDUCED ROOT NODULES. Catherine G. Beres, William Newcomb, Department of Biology, Queen's University.

LM and TEM studies of the spherical-shaped root nodules of garden bean (Phaseolus vulgaris), soybean (Glycine max), peanut (Arachis hypogaea), cowpea (Vigna unguiculata) have demonstrated the regular occurrence of two spherically-shaped structures, each 1 to 3 μm in diameter, which stain deep blue with toluidine blue O. In P. vulgaris the spherical bodies may have a dense granular appearance, are not membrane bound but may contain internal vesicles or may appear to be fusing with vesicles containing fibrillar or electron dense material. These structures only occur within infected cells. In 0.5 μm or ultrathin sections the usual number of these organelles is one but as many as three have been observed within a single host cell. In soybean, cowpea and peanut nodules, the spherical organelles resemble the cytoplasmic nucleoloids described in lily and cosmos pollen by J. Heslop-Harrison and H. Dickinson. The nucleoloids of nodules only occur within the cytoplasm of infected cells and are usually observed singularly in 0.5 μm or thinner sections. Cytoplasmic vesicles have not been observed to be associated with these organelles which may be surrouned by extensive circular profiles of rough ER. The nucleoloids of soybean and peanut nodules stain positively for RNA and protein.

LEAF SURFACE WAX OF CORN. Timothy W. Blaker, Department of Plant Sciences, University of Western Ontario.

Analysis of the surface wax extracted from individual leaves of a corn

(Zea mays cv. 'Ohio 43') plant at a given age indicates a transition in the composition of waxes found on seedling leaves as compared to that found on mature leaves. The waxes of seedling leaves (leaves 1-8) are dominated by the presence of large amounts of dotriacontanol (C_{32}-alcohol) and dotriacontanoic acid. The waxes of leaves present on the mature plant (ie. at anthesis) are a mixture of long-chain alkanes and also long-chain esters of alcohols and fatty acids. The amount of dotriacontanol is greatly reduced. There has been no indication of a change in composition on an individual leaf over time; there is an increase in the amount of wax extracted that is proportional to the increase in lamina area.

There are at least two possible explanations: 1) individual leaves have a genetically determined pattern of wax accumulation based on their order of ontogeny (heteroblasty); 2) as they originate at the meristem, individual leaves are influenced by environmental conditions (heat, water status, etc.), which produces the wax composition found on the leaves of the mature plant.